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USE OF BIOLOGICAL SYSTEMS FOR THE PREPARATION OF CHIRAL MOLECULES IV : A TWO-STEP CHEMOENZYMATIC SYNTHESIS OF A NATURAL PHEROMONE (4R,5S)-(-)-4-METHYL 5-HYDROXY HEPTAN 3-ONE, SITOPHILURE.

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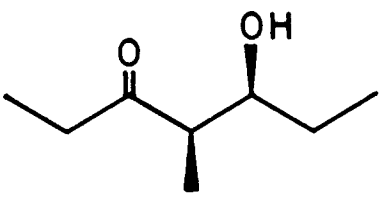
ABSTRACT : Reduction of 4-methyl heptan 3,5-dione 2 by resting cells of Geotrichum candidum provides natural sitophilure (4R,5S)-(-)-4-methyl 5-hydroxy heptan 3-one 1 under anaerobic conditions. Diastereoisomer (4S,5S)-(+)-4-methyl 5-hydroxy heptan 3-one 1a is obtained under aerobic conditions. Starting β-diketone is easily obtained by a one-pot synthesis. Good yield and high enantiomeric excess are obtained for the natural pheromone.

Among the versatile reactions with enzymes recently reviewed by Jones[1], asymmetric reduction of ketones, a major challenge in organic chemistry, is being thoroughly investigated[2]. As part of our continuing efforts in searching biological systems as tools for enantioselective synthesis, we studied this reaction on aliphatic ketones[3] and diones[4] with various microbial cells and purified alcohol-dehydrogenases. Some of our results, obtained for the synthesis of both enantiomers of a natural pheromone, led us to propose chemists a guide to choosing the most appropriate system for the reduction of any particular carbonyl compound[3].

In the present paper, we now report another biological reduction of a carbonyl group in which experimental conditions are the means to select, from a single cell, enzymes showing different enantiotopic face specificities. The synthetic application of this reaction is in the field of natural products, the reduced compound obtained being an insect pheromone named sitophilure, (4R,5S)-(-)-4-methyl 5-hydroxy heptan 3-one, 1.

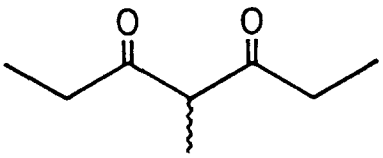


1

This aggregation-promoting compound is secreted by the adult male of both rice weevil (Sitophilus oryzae) and maize weevil (S. zeamais) which massively infest stored cereal grains. It has been extracted from insects, purified and characterized by Burkholder and coll[5]. Owing

to its biological and economic importance, amounts of this pheromone were needed to develop insect traps. As only micrograms were obtained from thousands of insects[5], chemical synthesis has been attempted. Starting from a single chiral source, Mori[6] was able to obtain all of the four possible stereoisomers of 4-methyl 5-hydroxy heptan 3-one required for biological assays. However, the over-all yields did not exceed 10 % and only 3 % of natural sitophilure was obtained after 13 steps. This prompted us to carefully study the biological reduction of a prochiral possible precursor of sitophilure, 4-methyl heptan 3,5-dione 2, in the course of our study on reduction of 2,4 and 3,5 diones by various yeasts[4,7] and fungi[7,8].

4-methyl heptan 3,5-dione



2

The β-diketone 2 was obtained by acylation of pentane 3-one with ethyl propionate by the sodium amide method[9] in a one-pot synthesis. The only micro-organism found to reduce this diketone is a yeast-like fungus Geotrichum candidum. All of our attempts of reduction by other microbial cells proved to be unsuccessful. Thus, trying to obtain different stereoisomers, we studied the reducing ability of this particular cell under various experimental conditions in which different enzymatic systems might be reached. We noticed that, like yeasts, G. candidum possesses, under anaerobic conditions, a fermentative metabolism. It could be assumed that the enzymes involved, in particular alcohol-dehydrogenases, might lead to compounds showing different stereochemistry than those obtain during the oxidative metabolism.

Bioconversion under aerobic conditions : the harvested cells of a 48-h old fermenter-grown culture of G. candidum[10] were carefully washed with saline and resuspended in glucose solution[11]. After 48 h of aerated incubation with the substrate, on a gyratory shaker at 27°C, cells were removed and the solution extracted with ether. GPC analysis of the residue showed that the starting dione has been entirely transformed into two compounds 1a and 1b showing closely related retention times and very different relative proportions (Table 1).

TABLE 1 : GPC* and ^{13}C NMR Data* for 4-methyl 5-hydroxy heptan 3-one diastereoisomers chemically and biologically obtained.

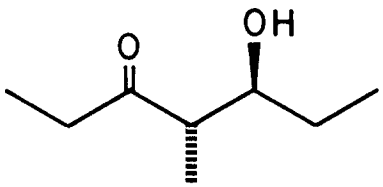
	R_t	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8
(±) anti (threo)[14]	4.9				50.7	74.9			13.9
(±) syn (erythro)[14]	5.3				49.7	72.8			10.2
Isomer 1a 90 % O_2, 10 % N_2	4.9	9.9	36.1	216.7	50.7	75	27.6	7.5	14.2
Isomer 1b 10 % O_2, 90 % N_2	5.3	10.0	35.1	216.5	49.5	72.8	27	7.6	10.4

* Retention times are in minutes and δ(ppm) are obtained for $CDCl_3$ solutions on a Bruker 300 MSL at 75.47 MHz.

Retention times were found to be identical to those obtained for racemic anti and syn diastereoisomers of 4-methyl 5-hydroxy heptan 3-one chemically obtained[12]. The major product having the shortest retention time has been purified by SiO_2 chromatography and analysed by ^1H and ^{13}C NMR[13]. According to Heackock's work on racemic syn and anti isomers[14], ^{13}C NMR data indicated that the reduced product obtained 1a is the anti isomer (Table 1).

As a dextrorotatory optical activity has been observed for the purified compound ($(\alpha)_J^{25}$ = +22°, c = 0.02 in ether[15]) its optical purity has been checked by 300 MHz ^1H NMR using a chiral shift reagent[16] and GPC analysis using a chiral phase capillary column[17]. Both techniques, requiring purified authentic racemic compound[18], showed an enantiomeric excess of 70 %. Chiral-phase GPC indicated that the major enantiomer (85 %) beared a C-5 carbon being S in absolute configuration. These results, in accordance with Mori who assigned the absolute configuration of the two anti enantiomers[6], indicate that the β-hydroxy ketone 1a obtained with G. candidum cells under aerobic conditions is the following compound :

(4S,5S)-(+)-4-methyl
5-hydroxy heptan 3-one 1a.

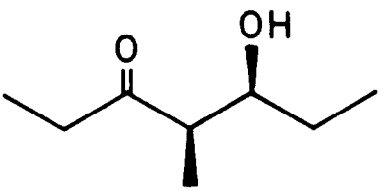


Bioconversion under anaerobic conditions : Anaerobic cofermentation of 4-methyl heptan 3,5-dione and various sugars was studied with washed, fermenter-grown, G. candidum cells[10]. Bioconversions were performed in serum bottles, flushed with nitrogen and sealed with septum caps[19]. After 48 h of incubation at 27°C on a gyratory shaker, the reactions were stopped by cell removal and ether extraction of the solutions. Results obtained by GPC analysis of the residues showed that whatever the sugar added (dextrose, saccharose, lactose) the reaction took place and no starting ketone remained, even in the proof bottles where no sugar had been added. As observed for aerobic reactions, two reduced compounds were detected by GPC, the major compound being, for all assays, the ketol with the longest retention time thus the syn isomer (Table 1).

An optical activity has been observed on the purified syn ketol 1b: $(\alpha)_J^{25}$ = -24°, c = 0.02 in ether[20]. ^1H NMR analysis using a chiral shift reagent[16] and GPC analysis on a chiral phase capillary column[17] showed that this product is enantiomerically pure and 100 % S in absolute configuration at C-5.

According to literature data[6], the syn ketol 1b obtained under anaerobic conditions is the natural sitophilure[21] 1:

(4R,5S)-(-)-4-methyl
5-hydroxy heptan 3-one 1b.



The results described above illustrate a new way of obtaining stereoisomers with biological systems. A single cell, placed under different conditions of aeration, can afford different enzymatic systems showing dissimilar stereoselectivity towards a given substrate.

At this point, the most that can be said is that under anaerobic conditions, many enzymes of the oxidative metabolism might be inhibited, in particular some alcohol-dehydrogenases involved in the bioreduction performed under aerobic conditions. Along with these enzymes, we noticed that enzymes leading to a further degradation of the product are inhibited under anaerobic conditions, as isolated yield of the reduced compound is better under nitrogen (70 %) than under oxygen (40 %). Work on this subject is in progress.

Anyhow, the simplicity and high regio- and enantio-specificities of this chemoenzymatic synthesis using microbial cells as catalyst, make it very convenient for a large-scale synthesis of the natural pheromone.

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REFERENCES AND NOTES.

1- J.B. Jones, Tetrahedron Report 203, Tetrahedron, 1986, 42, 3351
2- J.B. Jones, J.F. Beck, in Techniques in Chemistry, Wiley, New York, 1976, Vol.10, p.107 ; C.J. Sih, C.S. Chen, Angew. Chem. Int. Ed. Engl., 1984, 23, 57 ; E. Keinan, E.K. Hafeli K.K. Sethe, R. Lamed, J. Amer. Chem. Soc., 1986, 108, 162., and references there in.
3- A. Belan, J. Bolte, A. Fauve, J.G. Gourcy, H. Veschambre, J. Org. Chem., 1987, 52, 256.
4- J. Bolte, J.G. Gourcy, H. Veschambre, Tetrahedron Letters, 1986, 27, 565.
5- N.R. Schmuff, J.K. Phillips, W.E. Burkholder, H.M. Fales, C.W. Chen, P.P.Roller, M. Ma, Tetrahedron Letters, 1984, 25, 1533.
6- K. Mori, T. Ebata, Tetrahedron, 1986, 42, 4421.
7- A. Fauve, H. Veschambre, to be published.
8- A. Fauve, H. Veschambre, to be published.
9- R. Levine, J.A. Conroy, J.T. Adams, C.R. Hauser, J. Amer. Chem. Soc., 1945, 67, 1510.
10- Growth medium: Glucose 50 g, Yeast extract 10 g, Bacto peptone 10 g, Tap water 1 L.
11- 5g of wet cells and 50 µL of substrate in 50 mL of 5 % glucose solution per 500-mL erlenmeyer flask.
12- A.B. Smith, P.A. Lavenberg, Synthesis, 1981, 567.
13- ^{1}H NMR 300 MHz spectral values of 1a, δ(ppm) : 0.99 (t, J=7 Hz, 3H) ; 1.06 (t, J=7 Hz, 3H) ; 1.14 (d, J=7 Hz, 3H) ; 1.30 to 1.50 (m, 1H) ; 1.50 to 1.63 (m, 1H) ; 1.68 (s, 1H) ; 2.38 to 2.76 (m, 3H) ; 3.57 to 3.69 (m, 1H).
14- C.H. Heathcock, M.C. Pirrung, J.E. Sohn, J. Org. Chem., 1979, 44, 4294.
15- We noticed that ether is not a good solvent for optical rotation determinations because of its high volatility and its affinity for water. Optical rotation value is lower than the one observed by Mori even though its optical purity is in accordance with NMR analysis with chiral shift reageant. Moreover, when in solution in $CDCl_3$, 1a exhibits a levorotatory value : Lit. data[6] : $(\alpha)_D^{25}$ = + 36.8° c = 1.25 in ether.
Our data : $(\alpha)_D^{25}$ = + 22.0° c = 0.02 in ether. $(\alpha)_D^{25}$ = - 2.5° c = 0.024 in $CDCl_3$.
16- For this technique, ^{1}H NMR spectrum of the enantiomer recorded in presence of tris (3-(trifluoromethylhydroxymethylene)-d-camphorato) europium III is compared with the spectrum of the corresponding racemic obtained under the same conditions.
17- For more details on this technique see Ref. 4.
18- (±) anti hydroxy ketone has been separated from (±) syn isomer by column chromatography
19- 10 g of wet cells, 100 µL of substrate in 100 mL of deaerated 5 % sugar solution per 250-mL serum bottle. Nitrogen fluxed for 5 min. before sealing. Proof assays without sugar.
20- As already mentioned for 1a, optical rotation value of 1b is lower than the one published by Mori but ^{1}H NMR analysis with chiral shift reagent showed it to be optically pure. The solvent effect is remarkably strong as the reduced product obtained exhibits a dextrorotatory value in $CDCl_3$. Lit. data[6]: $(\alpha)_D^{25}$ = - 26.7°, c = 1,52 in ether.
Our data : $(\alpha)_D^{25}$ = - 24.0°, c = 0.02 in ether. $(\alpha)_D^{25}$ = + 32.0°, c = 0.014 in $CDCl_3$.
21- No elementary analysis has been performed, the products being already described.
^{1}H NMR 300 MHz spectral values of 1b, δ(ppm) : 0.96 (t, J = 7 Hz, 3H) ; 1.06 (t, J = 7 Hz, 3H) ; 1.14 (d, J= 7 Hz, 3H) ; 1.30 to 1.44 (m, 1H) ; 1.45 to 1.62 (m, 1H) ; 2.40 to 2.70 (m, 3H); 2.77 (s, 1H) ; 3.78 to 3.87 (m, 1H).

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